BESTCHROM
博 格 隆

IMAC Bestarose FF Metal chelate chromatography resin

Instruction for use

Contents

1. Introduction

IMAC Bestarose FF(Fast flow)is a metal chelating affinity chromatography resin, which can be widely used to separate and purify proteins and peptides. The principle is that the side chains of histidine, cysteine and tryptophan of protein interact with a variety of transition metal ions such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, so as to achieve the purpose of separation and purification.

IMAC Bestarose FF is crosslinked by cross-linked agarose and aminotriacetic acid (NTA). It can chelate the four price points of metal ions, make the chelated metal ions more stable, tolerate higher reducing agents, have good physical and chemical stability, and have advantages in good specificity and fast flow velocity.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Cross-linked agarose, 6%
Particle size +	45-165μm
Metal chelating capacity	Ni^{2+}/ Zn^{2+}：15μmol/mL resin，Cu^{2+}：25μmol/mL resin
Dynamic binding capacity	Ni^{2+}：～40mg His tag protein/mL packed resin
Chemical stability ++	1 week at 40℃: 10mM HCl, 0.1M NaOH, 8M urea, 6M GuHCl; 40℃ 12h: 1M NaOH, 70% acetic acid;
pH stability+++	3~12(working) 2~14 (CIP)
Pressure flow velocity	~600cm/h（0.1MPa BXK16/20 H=5cm 25℃）
Max. pressure	0.3MPa
Storage++++	2~30℃,20% ethanol or 2% benzyl alcohol
Recommended flow velocity	＜150cm/h

+ The particle size is normally distributed, and the particles in this range account for more than 95% of the total

++ Stability when removing metal ions

+++ CIP refers to pH stability when metal ions are removed.

++++2% benzyl alcohol is only used for international transport or special requirements from customer

3. Method of chromatographic

3.1 Column packing

Note：It is best to equilibrate the resin slurry to room temperature before column packing.

- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.15 (Compression factor=1.15)

  According to the volume of the settlement resin required, the suspended slurry of the resin required is calculated by the follow:

  Required resin slurry[1] volume = Settlement resin volume ÷ Resin slurry [1] concentration. The original concentration of resin slurry [1] is shown in the follow table.

Pack size	Resin slurry [1] concentration (%)
25mL、100mL、500mL、1L、5L、10L	80
20L、40L	75

1: It refers to the original packaging resin slurry sold by Bestchrom.

Note: For non-original packaging, customer can calculate the required volume according to the actual concentration of resin slurry.

- Washing the resin: Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain a 50%~75% slurry, stir well and set aside for use.
- Take a cleaned BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Purge the bubbles trapped at the end-piece net by draining some distilled water through the column outlet. Leave about 1cm water at the bottom of the column and close the bottom outlet. Adjust the column so that it is perpendicular to the ground.
- Slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

  Packing reservoir: Empty glasstube with same diameter as the BXK column.

- Fill the remainder of the column with packing solution. Connect the packing reservoir to the chromatography system, open the flow velocity, drain the bubbles in the hose, close the flow velocity, and tighten the top cover of the packing reservoir.

✧ After pouring, stir well again with Stirrer, and then wash the resin particles on the inner wall of the column from top to bottom with the packing solution, and let the resin settle naturally until there is about 1cm of clarifying solution on the suspension. Mount the adapter and connect the adapter to the chromatography system or peristaltic pump. Lower the adapter to descend to

contact with the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the outlet of the top piece is opened, slowly move the adapter down until all bubbles are drained.

Note: This operation is only applicable to BXK 100 and above columns. Flushing the inner wall reduces the resin particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- When the bed height is 10cm, the flow velocity can be set to 75cm/h. Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized, mark the bed height.
- Remove the packing reservoir (if any), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity at 260cm/h, and continue to press the column using the above flow velocity until the bed is completely consolidated, mark the consolidated bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.2 Evaluation of Packing

- The packing quality of chromatographic column can be confirmed by column efficiency measurement and evaluation. The tests are required after the column packing, during the column working life and when the separation and purification performance weakens. The method usually relies on the height equivalent to a theoretical plate(HETP) and the asymmetry factor(As).
- Acetone or NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	Acetone method	NaCl method
Sample	1.0% (v/v)acetone in water	0.8M NaCl in water
Sample volume	1.0%CV	1.0%CV
Mobile phase	Water	0.4M NaCl in water
Flow velocity	30cm/h	30cm/h
Monitor	UV280 nm	Conductivity

- Method for measuring HETP and As:

  Use UV curve or the conductivity curve to calculate the height equivalent of theoretical plate (HETP), number of theoretical plates(N) and the asymmetry (As):

  HETP=L/N

  $N=5.54(V_R/W_h)^2$

  Note: V_R = retention volume

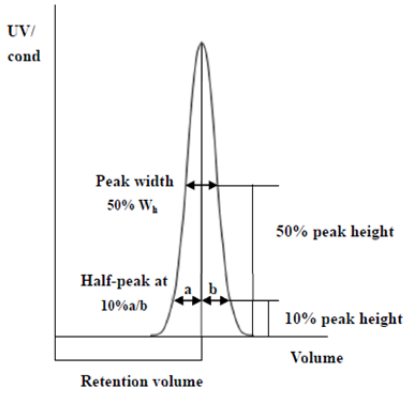
  W_h = half-peak width

  L = column height

  N = the number of theoretical plates

  (The units of V_R and W_h should be the same)

  As=b/a



Note:

a= 1st half peak width at 10% of peak height

b= 2nd half peak width at 10% of peak height

- Evaluation the column packing

  As a guideline, if the value of HETP is less than 3 times the average particle size(d_{50}) of the resin and the As is between 0.8~1.8, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked.

3.3 Chromatography method

- Chelate metal ions：
- Rinse 5CV with purified water，
- Rinse 5CV with equilibration buffer
- Rinse 5CV with purified water
- Use 5CV of 100mM metal ion solution through the chromatography column
- Unbound metal ions were removed with a 10CV balancing buffer
- Rinse 10CV with purified water
- The chromatography column was cleaned with 10CV elution buffer
- Equilibrate the chromatographic column with equilibration buffer and wait for use.

  The general metal ion environment is neutral (pH 7-8). Zinc ion selects pH≤5.5 to avoid the solubility of high pH salt; iron ion selects pH≈3 to avoid the formation of insoluble matter.

- Buffer: Phosphate buffer is preferred, the pH range is neutral (between 7-8), avoid using EDTA and citrate, etc. Tables 1 and 2 list common additives and concentrations that do not affect or affect metal chelation chromatography.

  In order to reduce the non-specific binding of the host protein to the resin, low concentrations of imidazole (20-40 mM) are usually added to the equilibration buffer and the sample.

  NaCl of 0.15 ~ 0.5M must be added to the buffer solution to eliminate ion adsorption.

Table 1 Additives that do not affect protein binding to immobilized metal ion affinity resin

Additives	Concentration	Additives	Concentration
Phosphate、Tris、Borate、HEPES	20-100mmol/L	Non-ionic detergent	2%
NaCl	2mol/L	Triton X-100	2%
KCl	1mol/L	Tween-20	2%
Guanidine hydrochloride	6mol/L	Octyl glucoside	2%
Urea	8mol/L	Dodecyl maltoside	2%
glycerin	50%	$C_{12}E_8$,$C_{10}E_6$	2%
Isopropanol	60%	PMSF (Protease inhibitor)	1mmol/L
Ethanol	30%	Pepsin inhibitor	1μmol/L

		(Pepsin inhibitor)	
Amphoteric detergent (CHAPS)	1%	Leupeptin (Protease inhibitor)	0.5μg/mL
1% Benzamidine (Protease inhibitor)	1mmol/L	/	/

Table 2 Additives that may disrupt protein binding to the immobilized metal ion affinity resin

Additives	Concentration	Additives	Concentration
2-mercaptoethanol	20mmol/L	Histidine	Can be used instead of imidazole
Strong reducing agents (DTT and DTE)	0.1mmol/L	Glycine	—
Chelating agents (EDTA and EGTA)	0.1mmol/L，take Ni^{2+} from the resin	Glutamine	—
Ionic detergent (cholate, SDS)	—	Arginine	—
Sodium azide	3mmol/L	Ammonium chloride	—
Citrate	Can tolerate low concentrations	—	—

- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer. The loading volume is determined according to the substance content in the sample and the binding load of IMAC Bestarose FF.
- Equilibrium: The equilibrium buffer is used to clean the chromatography column until the pH, conductivity and UV of the effluent are the same as that of the equilibrium liquid phase.
  In order to reduce the impact of metal ion shedding on chromatography, it is recommended to clean 0.5M imidazole containing 1M NaCl with 1CV before balancing, then clean 5CV with purified water, and finally balance the chromatography column with balancing buffer before loading samples.
- Sampling: Sample the prepared samples according to the set conditions.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution:
  - Competitive elution: linear increase or one-step increase of substances with affinity for metal ions, such as 0-0.5M imidazole, 0-2M NH_4Cl. Gradient elution is best performed at a constant pH in equilibration buffer.

    The pH of the buffer can be lowered for elution. When the pH of the buffer is lower than 4, metal ions will dissociate with the resin to achieve the purpose of elution. (If the target protein is

sensitive to low pH, it is recommended to add 1/10 volume of 1M Tris-HCl to the eluted collection solution, pH 9.0 for neutralization)

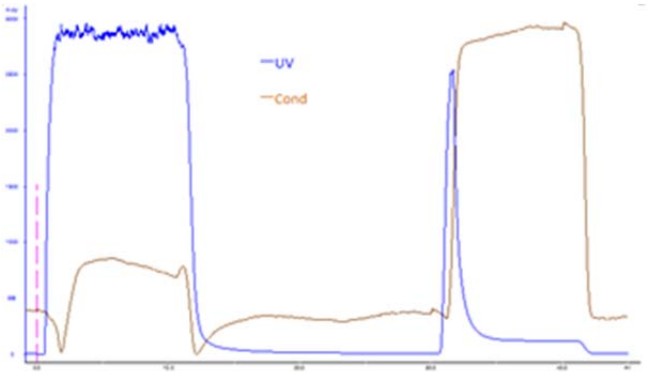
The chelating agents EGTA and EDTA can dissociate metal ions from the resin to achieve elution, and the metal ions in the elution products can be removed by desalting column.

In order to reduce the impact of metal ion shedding on chromatography, it is recommended to clean 0.5M imidazole with 1CV before balancing, then clean 5CV with purified water, and finally balance the chromatography column with balancing buffer before loading samples.

- Regeneration:Impurity residue and shedding of metal ions will affect the column's chromatographic performance and loading capacity.It is recommended that metal ions be re-chelated after every one to five cycles according to production needs.
  - Nickel was removed with 2~5CV of buffer solution (50mM PB, 0.5M NaCl, 0.1-0.2M EDTA, pH 7.0); Fe^{3+} is easy to form insoluble matter in neutral solution, so it is recommended to use 50mM EDTA overnight to remove metal ions.
  - The residual EDTA was removed by 2~3CV of 0.5M NaCl passing through the column.
  - 0.5CV of 0.2M metal ion solution was used to pass through the chromatography column
  - Remove unbound metal ions with 5CV purified water;
  - The chromatography column was cleaned with 5CV tric elution buffer;
  - Balance the chromatography column with a balancing buffer and set aside.

4. Application

Application of IMAC Bestarose FF in purification of histidine-tagged recombinant protein



Column：EzFast 1mL
Bed height：2.5cm
Buffer solution：A: 25mM imidazole + 0.15m NaCl pH: 7.00
B: 500mM imidazole pH: 7.00
Sample：*E.coli* expression was recombinant with His-GST label
Loading quantity of sample：Supernatant of protein lysate 10mL
Flow velocity: 1mL/min

5. Cleaning-in-place(CIP)

With the increasing use of chromatography resin, the accumulation of contaminants on the chromatography column is also increasing. Cleaning-in-place can prevent the accumulation of contaminants and maintain a stable working state. Determine the frequency of CIP according to the degree of contamination of the resin (if the contamination is serious, CIP should be carried out after each use to ensure repeatability of the results).

- First remove nickel ions;
- To remove the protein adsorbed by ion exchange: wash the column with 2M NaCl solution of 2-3 times the bed volume, and then wash the column with distilled water of 3 times the bed volume;
- Precipitated or denatured material: can be removed with 1M NaOH for 0.5-1h;
- Hydrophobic binding substance: 2CV 70% ethanol or 30% isopropanol to wash the column, immediately do reverse washing with at least 5CV of filter-sterilized equilibration buffer.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that IMAC Bestarose FF can be treated with 70% ethanol for more than 12h before use or during use, or the resin after nickel removal can be treated with 1M NaOH for 0.5-1h to reduce the risk of microbial contamination.

7. Storage

IMAC Bestarose FF is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

8. Disposal and Recycling

IMAC Bestarose FF is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
IMAC Bestarose FF	AA0061	25mL
	AA0062	100mL
	AA207311	500mL
	AA0063	1L
	AA0064	5L
	AA0065	10L
	AA207315	20L

Prepacked columns	Code No.	Pack size
EzFast IMAC FF	EA207301	1×1mL
	EA207303	1×5mL
	EA009	5×1mL
	EA010	5×5mL
EzScreen IMAC FF	EA00625	1×4.9mL
	EA00635	5×4.9mL
EzLoad 16/10 IMAC FF	EA207304	1 pcs
EzLoad 26/10 IMAC FF	EA207306	1 pcs